MICROBIOLOGY

Project title: **Bacteria Living at Low pH and High Temperature**

Principal investigator: Dr. Rick Bizzoco
Phone number: 619-594-5396
Email: *rbizzoco@sunstroke.sdsu.edu*
Address: Department of Biology
San Diego State University
5500 Campanile Drive
San Diego, CA 92182-4614

Objective: Discovery of new hyperthermal acidophiles.

Findings: We have identified several new organisms in our electron microscopic analysis. Phase-contrast microscopy has revealed numerous morphological types in the hot springs we have examined. DNA staining was performed to document that the forms observed indeed were biological entities and/or living microbial cells. We compared these findings with those obtained in 1971 while working with Professor T. Brock.

Project title: **Transition between Lithoautotrophy and Chemoheterotrophy in *Sulfolobus* species**

Principal investigator: Dr. Paul Blum
Phone number: 402-472-2769
Email: *pblum@biocomp.unl.edu*
Address: E234 Beadle Center
School of Biological Sciences
University Nebraska-Lincoln
Lincoln, NE 68588-0666

Objective: To determine the factors which regulate the metabolic status of cells in situ.

Findings: None within the year 1999.

Project title: **Molecular Ecology of Photosynthetic Hot Spring Bacteria that Resemble *Heliothrix oregonenesis***

Principal investigator: Dr. Sarah Boomer
Phone number: 503-838-8209
Email: *boomers@wou.edu*
Address: Department of Biology
Western Oregon University
345 Monmouth Avenue
Monmouth, OR 97361

Objective: The goal of this research is to survey thermal areas of Yellowstone for new representatives of hot spring bacteria that resemble *Heliothrix oregonensis*, a photosynthetic bacteria that has been described in alkaline hot springs in the Oregon Cascades. Evidence from other labs and ours suggests that *Heliothrix*-like organisms are prevalent in similar habitats in Yellowstone National Park. Using DNA/molecular methods, we isolate bacterial DNA from new mat samples containing *Heliothrix*-like bacteria, as suggested by habitat, microscopic, and photosynthetic pigment analysis. From this DNA, we amplify and sequence 16S rRNA genes, genes used for identification purposes. Sequences obtained are compared to national DNA databases and used to identify and classify novel strains, thus determining the precise relationships between a) the new organisms and *Heliothrix*; or b) different isolates within Yellowstone National Park.

Findings: Over the summer of 1999, five undergraduates and I surveyed four distinct areas, performing habitat analysis in the field. Of these, we ruled out three areas based on habitat parameters (Canyon/Seven Mile Hole and Factory Hill/Columbia group, both near Heart Lake) and removed samples from one mat in the Witch Creek drainage near Heart Lake. Further microscopic and pigment analysis of this site following the field trip confirmed the presence of *Heliothrix*-like bacteria. We also surveyed and sampled three previously sampled sites (Spray/Fairy and Hillside) in order to document mat growth/recovery following the 1998 trip and collected material so as to determine the amount of genetic variability that occurs over time in these important sites. Presently, two undergraduates are characterizing 16S rRNA clones from the Hillside-1999 samples. To date, they have twenty-four different clones and are in the process of sequencing them.

Project title: **Recognizing the Signatures of Hyperthermophilic Biofilms within Hydrothermal Ecosystems and their Fossilized Deposits**

Principal investigator: Dr. Sherry Cady
Phone number: 503-725-3377
Email: *CadyS@pdx.edu*
Address: Portland State University
Department of Geology
17 Cramer Hall, 1721 SW Broadway
Portland, OR 97207

Additional investigators: Zach Oestreicher, Liz McKenzie

Objective: To understand how hyperthermophilic communities that occupy modern hydrothermal ecosystems are preserved in the rock record in order to enhance our ability to properly interpret their fossilized counterparts. Given that hydrothermal deposits are targets for future Mars missions, we are establishing criteria for the detection and proper interpretation of hyperthermophilic biosignatures found in a variety of different types of siliceous sinters. The strategy relies on characterizing the biogeochemical signatures of modern hyperthermophilic communities using a combination of field, experimental, and analytical techniques (i.e., quantitative mineralogy and petrography, electron beam microscopy and chemical analysis, isotopic and biomarker compound analysis, and, in collaboration with N.R. Pace's group, molecular phylogenetic identification).

Findings: We have identified that although organically preserved hyperthermophilic microfossils are rarely preserved in high temperature siliceous sinter deposits, hyperthermophiles are preserved as microfossils by other processes. Our findings indicate that the characteristics of the organisms and the geochemical conditions of the surrounding environment play a role in determining the fidelity of preservation of morphological biosignatures. We are currently using a variety of optical and submicroscopic hybridization probes to better understand the exact mechanisms responsible for the preservation of the hyperthermophilic biosignatures that we have observed in siliceous sinters. In collaboration with the Pace Lab, we continue to document the biogeographical distribution of hyperthermophilic organisms in hot springs and geysers. We have also discovered that differences in the architecture of hyperthermophilic biofilms can be related to differences in the microstructure of siliceous sinters, and we are investigating the potential for sinter microstructures to serve as important biosignatures.

Project title: **Adaptations of Cyanobacteria to High Solar Irradiance: UV Tolerance Strategies**

Principal investigator: Dr. Richard Castenholz
Phone number: 541-346-4530
Email: *rcasten@darkwing.uoregon.edu*
Address: Department of Biology
University of Oregon
Eugene, OR 97403-1210

Additional investigators: Jesse Dillon, Scott Miller

Objective: One objective was to evaluate the role of the UV-absorbing pigment, scytonemin, in increasing the fitness of cyanobacteria exposed to high solar irradiance. A second objective was to determine if the exclusion of UV radiation over a hot spring mat composed of several species of cyanobacteria for a two month period would result in changes in species composition, i.e., allow less UV-resistant species to become dominant.

Findings: One study measured differences in responses to UV in two hot spring populations of the same species of cyanobacteria. In the comparison between these two streams, differences in growth and UV responses were observed. The evidence suggests that an unknown toxic substance is present in one of the streams that is inhibiting growth and promoting the production of the UV-screening pigment, scytonemin, compared to the sister population in the other stream. The presence of scytonemin was correlated with a lower percent inhibition by UV radiation, as compared to the same species with no scytonemin in Rabbit Creek. In another related study, populations of other cyanobacteria in two hot spring streams were subjected for two months under UV-excluding filters. Results indicate that there was no significant change in composition unless total irradiance was decreased.

Project title: **Heat Stable Enzymes from Thermophiles**

Principal investigator: Dr. Joan Combie
Phone number: 406-388-0942
Email: *mtbiotech@montana.net*
Address: Montana Biotech Corporation
1740 East Baseline Road
Belgrade, Montana 59714

Additional investigators: Fred G. Albert

Findings: Eight samples were collected for a student at the University of Warwick working under Dr. Paul Norris. No other work was done in Yellowstone or on heat stable enzymes in 1999.

Project title: **Isolation and Characterization of Microorganisms Extremely Resistant to DNA Damage**

Principal investigator: Dr. Jocelyne DiRuggiero
Phone number: 410-234-8890
Email: *Diruggie@umbi.umd.edu*
Address: Center of Marine Biotechnology
University of Maryland
701 East Pratt Street
Baltimore, MD 21202

Additional investigators: Frank T. Robb

Objective: Very little is known about strategies adopted by extremophiles to maintain the integrity of their genetic material in very hot environments. For all cells to survive, they must continuously and accurately repair lesions to their DNA caused by environmental stress. For hyperthermophiles, additional damages are inflicted on their DNA by exposure to elevated temperature. In preliminary studies, we have found that the hyperthermophile *Pyrococcus furiosus* is extremely resistant to ionizing radiation. Based on these observations, we propose the following hypothesis: "Hyperthermophiles' resistance to ionizing radiation is due to their unusual ability to repair extensive heat induced DNA double-strand breaks, which occur at a much higher rate at elevated temperature. Therefore, hot environments should also be a prime source of highly radioresistant microorganisms."

The proposed project addresses the following questions: What are the highest temperatures under which genome integrity can be maintained? How do hyperthermophiles protect and repair their DNA? To address these questions, we will isolate and characterize novel and highly resistant thermophiles from hot springs in Yellowstone National Park containing elevated radon levels and exposed to high fluxes of solar radiation. In addition, we will use extreme UV- and g-irradiation as selective pressure during enrichment to eliminate competing radiation sensitive microorganisms. We will investigate the accumulation of DNA lesions from exposure to sublethal doses of radiation and the kinetics of removal of those lesions with the most radiation resistant isolates. We will assess the performance of the new isolates under simulated space conditions at NASA Goddard Space Flight Center and at the National Institute of Standard and Technology Synchrotron facility. Long-duration tests will determine the performance limits of the isolates exhibiting the greatest survival potential. In addition, survival and recovery of microbial isolates will be measured after their exposure to full spectrum solar radiation during a Solar Extreme Ultraviolet Rocket Telescope and Spectrograph (SERTS) flight.

Findings: We have completed our sampling expedition to Yellowstone National Park. Samples spanning a wide range of temperature, pH, and UV-radiation exposures have been collected. Physico-chemical parameters such as temperature, pH, and radon levels were compiled for each sampling site. Radon was measured in spring water using a a-scintillometer and the technique previously described by our collaborator Dr. M. Reimer. For each sampling site, GPS measurements and digital pictures were also accumulated. We are currently conducting enrichment cultures for the isolation of microorganisms from our

Yellowstone samples. A wide variety of culture media, solid and liquid, have been inoculated with water and sediments collected in hot springs of the park. Incubation temperature varies from 65 to 98° C in anaerobic and aerobic conditions. We are particularly interested in *Deinococcus/Thermus* types of microorganisms that have the potential to be extremely radiation resistant and to grow at high temperature. Prior to further isolation, we are also submitting enrichment cultures to desiccation, UVC, and g-irradiation. The end products will be compared to control enrichment which have not been treated. Five purified cultures have already been obtained and growth curves and partial 16S rRNA sequences are being established for these microorganisms.

In parallel, we have also started testing control microorganisms for hard vacuum conditions. The following cultures have been exposed to hard vacuum in the SERTS instrument at the GSFC: 1) *Deinococcus radiodurans*, growing optimally at 30° C, the most ionizing radiation resistant organism known; 2) *Pyrococcus furiosus* growing optimally at 100° C and extremely resistant to g-irradiation; and 3) dormant spores of *Actimomycete* strains CHR3 and CHR28. The cells were desiccated and exposed to the SERTS vacuum for 4 days. We are currently evaluating the survival of those cells, compared to control cells that have only been desiccated and maintained at atmospheric pressure.

Project title: **Biosedimentology, Microbiology, and Geochemistry of Modern Hot Springs**

Principal investigator: Dr. Jack D. Farmer
Phone number: 480-965-6748
Email: *jfarmer@asu.edu*
Address: Arizona State University
Department of Geology
P.O. Box 871404
Tempe, AZ 85287-1404

Additional investigators: David J. Des Marais, Linda Jahnke

Objective: The objective of our research is to carry out integrated microbiological and geochemical studies of stromatolite-forming microbial mat systems in modern and ancient hot springs over a broad range of spring temperatures and compositions. Specifically, we wish to understand the factors that control the fossilization and long-term preservation of microbial biosignatures in hot spring sinters. Toward that end, we have been characterizing modern sinter deposits and their associated microbiotas over a broad range of temperatures, pH conditions, and spatial scales. This work provides a basis for comparing ancient thermal spring sinters from localities within Yellowstone National Park, as well as other hydrothermal areas of the world. The key questions in modern-ancient comparisons center around the long-term retention of microbial biosignatures during early and late diagenesis, and the geological and paleontological record of Precambrian hydrothermal environments and their evolution.

Findings: We have described the major and micro-scale features of terraced travertine deposits and

their biosedimentology based on comparative studies at Mammoth Terrace and Gardiner. We have also conducted detailed geochemical studies (stable isotopes, major and minor element variations) at Angel Terrace in the last year and now have obtained similar data for Hillside Springs. We have completed our initial taphonomic studies of microbial mats in siliceous and travertine springs. This included measuring microprofiles in oxygen, pH, and sulfide for microbial mats at Angel Terrace, Fountain Paint Pots, and Octopus Springs. Next year or so we intend to complete geochemical studies of ancient travertines (Highland Terrace and Gardiner), explore for ancient siliceous spring deposits in the Artist Point area, complete mineralogical studies at Hillside Springs, and begin collaborative work on mat biogeochemistry.

Project title: **Protein Comparison of Thermophiles and Oral Bacteria**

Principal investigator: Dr. Richard Gregory
Phone number: 317-274-9949
Email: *rgregory@iusd.iupui.edu*
Address: Indiana University
1121 Michigan St.
Indianapolis, IN 46202

Objective: Oral bacterial microflora are extremely diverse (more than 300 different species in the normal oral cavity) and have to survive relatively large temperature and nutritional variations. Thermophilic microorganisms have been fairly well described, but no comparison has been reported with oral bacteria. It is proposed here to compare protein antigens between thermophilic and oral bacteria by immunological and electrophoretic (protein size) techniques. SDS-PAGE electrophoresis will be used to compare the sizes of proteins between representative thermophiles and laboratory strains of oral streptococci (primarily *Streptococcus mutans*, the causative agent of human dental caries). Immunological assays such as ELISA and western blots will be used to compare reactivity between antibodies to protein antigens on *S. mutans* and the thermophiles. It is anticipated that similar proteins will be observed between thermophiles and oral bacteria implying a possible common ancestry.

Findings: Bacterial colonies were isolated on both selective and non-selective petri plates. Selected colonies were propagated and stored frozen until assayed. Samples are currently being collected from human volunteers to compare to park samples. It is anticipated that more park samples will be required due to poor growth of several bacterial colony types upon secondary propagation.

Project title: **Analysis of a Eukaryotic Microbial Mat Community across Environmental Gradients in a Thermal Acidic Stream**

Principal investigator: Dr. Joan Henson
Phone number: 406-994-4690
Email: *jhenson@montana.edu*
Address: Department of Microbiology
Lewis 109
Montana State University
Bozeman, MT 59717

Additional investigators: Michael Ferris, Kathy Sheehan, Keith Cooksey

Objective: To investigate the eukaryotic genetic diversity, ecophysiology, and behavior of a mat community in Nymph Creek using contemporary molecular analysis and microscopic methods with particular interest in the interaction between *C. caldarium* (a single-celled alga) and *D. constricta* (a filamentous fungus).

Findings: Preliminary microscopic examination has begun. PCR clone libraries are being investigated.

Project title: **Comparative Study of Sulfur-Turf Microbial Mats between Japanese Hot Springs and Yellowstone**

Principal investigator: Dr. Kenji Kato
Phone number: +81-263-37-2394
Address: School of Allied Medical Sciences
Shinshu University
Matsumoto 390-8621
Japan

Additional investigators: Hiroyuki Yamamoto, Yunosuke Maki

Objective: To evaluate the genetic difference between the bacteria in Japanese sulfur-turf and those in Yellowstone's. Sulfur-turfs exist in hot spring water effluent when the temperature exceeds 50° C and the water contains a significant amount of hydrogen sulfide. From analysis of small sub-unit rRNA of sulfur-turf taken from Japanese hot springs, we found that sulfur-turf bacteria are genetically very ancient. Thus, knowing the genetic difference between Japanese sulfur-turf bacteria and those of Yellowstone is an exciting subject, which will lead to an idea of the cause of biodiversity and evolution.

Findings: We found sulfur-turf-like bacteria from two sites in Yellowstone and started to analyze their genetic similarity with Japanese sulfur-turf bacteria. We are continuing the analysis.

Project title: **Population Biology of *Sulfolobus* spp. in Acidic Hydrothermal Environments**

Principal investigator: Dr. Brian Kinkle
Phone number: 513-556-9756
Email: *kinkleb@email.uc.edu*
Address: Department of Biological Sciences
University of Cincinnati
P.O. Box 210006
Cincinnati, OH 45221-0006

Additional investigators: Dennis Grogan

Objective: 1) To isolate and characterize anaerobic, pyrite-forming bacteria. 2) To isolate and characterize thermophilic, acidophilic archaea.

Findings: We have isolated several Gram-positive and Gram-negative bacterial strains capable of anaerobic formation of pyrite. We are currently characterizing the physiology of these strains and the minerological effects of its activities. In addition, we have isolated, characterized, and archived numerous thermophilic, acidolophilic archaea and are currently designing molecular criteria for differentiating among them.

Project title: **Microbial Biotransformations and Ecology**

Principal investigator: Dr. Charles Kulpa
Phone number: 219-631-5592
Email: *kulpa.1@nd.edu*
Address: CEST
152A Fitzpatrick Hall of Engineering
University of Notre Dame
Notre Dame, IN 46556

Additional investigators: Mark A. Schneegurt, Sophia Y. Dore

Objective: To isolate microorganisms with unique metabolic activities allowing for the transformation of polycyclic aromatic compounds that may contain heterocyclic rings and related products from the petroleum industry.

Findings: Samples obtained from the park in July 1999 have been used as inocula for enrichment cultures. The compounds of interest were provided as sole carbon, energy, or sulfur sources. Cultures were maintained aerobically under mesophilic or thermophilic conditions using a variety of media. Selection of interesting organisms from the enrichment cultures will continue through 2000. Soil

samples taken for analysis of microbial community structures in hot spring sediments have yet to be studied and remain in a freezer repository. A few soil samples have been included in a survey of soils used for testing DNA extraction procedures. Two roadside soil samples are being tested for platinum group elements. New enrichment cultures may be started if fresh inocula are obtained in 2000.

Project title: **Bacterial Diversity of Thermophilic Photosynthetic Bacteria**

Principal investigator: Dr. Michael T. Madigan
Phone number: 618-453-5130
Email: *madigan@micro.siu.edu*
Address: Department of Microbiology
Mailcode 6508
Southern Illinois University
Carbondale, IL 62901-65

Objective: The main objective of this research is to discover and isolate laboratory cultures of anoxygenic (non oxygen-evolving) photosynthetic bacteria from thermal environments. Photosynthetic bacteria are model organisms for the study of basic problems in photosynthesis, and thermophilic phototrophs are very desirable because of their thermostable photosynthetic machinery. The long-term goal of the research is to probe photosynthetic diversity in hot springs of various chemistries and temperatures to determine the physiochemical limits to photosynthesis. This includes isolating and characterizing new species of photosynthetic bacteria and studying their basic biological properties including physiology, biochemistry, and phylogenetic position in laboratory cultures. All cultures of thermophilic phototrophs from Yellowstone as well as New Zealand thermal springs have been deposited in the American Type Culture Collection (ATCC) for public access by any qualified individual. This is basic research; no commercial funding or research ties exist between this project and any for-profit organization.

Findings: No sampling was done in Yellowstone National Park during calendar year 1999. However, hot springs were sampled in Rotorua, New Zealand. These are springs that contain the thermophilic green sulfur bacterium *Chlorobium tepidum*. Previous searches for strains of this organism in YNP have been unsuccessful. However, using specific enrichment culture protocols that have been successful with New Zealand samples, we continue attempts to document this photosynthetic bacterium in microbial mats that form in high sulfide, slightly acidic hot springs in YNP, such as those in the Mammoth Upper Terraces region. Work also continues on the development of specific nucleic acid probes for use in surveying hot spring microbial mats for the presence of all four major groups of photosynthetic bacteria.

Project title: **A Molecular Analysis of the Microbial Diversity present in the Greater Yellowstone Ecosystem**

Principal investigator: Dr. Eric Mathur
Phone number: 619-623-5141
Email: *emathur@diversa.com*
Address: Diversa Corporation
10665 Sorrento Valley Road
San Diego, CA 92121

Additional investigators: Martin Keller, Jay Short, Terrance Bruggeman

Objective: Diversa's research efforts are directed towards gaining a better understanding of the microbial diversity present within Yellowstone National Park; coupled with this molecular taxonomic survey, Diversa scientists will employ recombinant techniques to screen environmental samples for relevant biomolecules. Attempts will then be made to correlate phylogenetic and catalytic diversity.

Findings: During FY97, Diversa scientists collected environmental samples from the following regions within Yellowstone: Heart Lake, Norris Geyser Basin, Octopus Springs, Five Sister Springs and Obsidian Pool. Nucleic acids were isolated from many of these samples and some have been captured in the form of 16S and environmental DNA libraries. Work in progress includes sequencing of unique 16S clones and construction of phylogenetic trees, as well as ongoing screening of the environmental libraries for clones expressing a variety of enzymatic activities.

Project title: **An Analysis of Soil Microbial Community Structure in an Evolving Thermal Soil Environment**

Principal investigator: Dr. Timothy McDermott
Phone number: 406-994-2190
Email: *timmcder@montana.edu*
Address: Montana State University
Department of LRES
334 Leon Johnson Hall
Bozeman, MT 59717

Additional investigators: Tracy Norris, Jon Wraith

Objective: The objective of this work is to use molecular methods to analyze soil microbial community succession in response to changes in soil temperature. Investigations of the biology of hydrothermal systems have added greatly to our understanding of microbial species diversity and their evolutionary relationships. However, previous studies have generally been limited to thermal systems that are well established on the time scale of human observation. The death of lodgepole pines in this study site is

indicative of a very recent expansion of the underlying geothermal plumbing. In some places temperatures as high as 80º C were recorded, which only six months previously were closer to 25º C. This study site provides us with a unique opportunity to observe changes in microbial community structure as they occur. This work will allow us to address questions concerning the forces affecting microbial community structure, diversity, and the colonization of geothermal features by thermophilic microorganisms.

Findings: A research plot was designated and thermocouple probes were inserted in the ground at specific locations within the plot to measure soil temperature at regular intervals. Temperature data collection was initiated in November 1999. Results indicate that the research plot includes an area of expanding geothermal activity. Soil samples were collected at several sites within the research area. Extraction of nucleic acids (DNA and RNA) from these samples by conventional protocols is underway in the lab. The DNA extracted from soil samples was PCR (polymerase chain reaction) amplified using primers specific for ribosomal gene sequences of bacteria. The PCR products were then subjected to Denaturing Gradient Gel Electrophoresis (DGGE) to compare the bacterial diversity of the sampling sites. Each band on a DGGE gel represents a unique DNA sequence, which in theory corresponds to a unique organismal species. Initial results suggest that temperature has been a selective force in thermally impacted soils as evidenced by apparent reduced species diversity from these sites.

Project title:	**Characterization of the Microbial Rhizosphere Population of Acid and Thermotolerant Grasses associated with Hot Springs and Microbial Diversity in Thermal Soils in YNP**
Principal investigator:	Dr. Timothy McDermott
Phone number:	See previous entry
Additional investigators:	B. Inskeep, M. Burr, M. Young, L. Botero

Objective: To study the diversity and identification of the thermophilic and acidophilic organisms associated with thermophilic plants located in YNP. We are also very interested in examining the diversity of the microbial community that thrives in select thermal soil locations.

Findings: We have obtained molecular evidence that some thermal soils (65°C to 85°C) apparently have diverse and complex prokaryotic communities. One pure culture isolate appears to represent a new taxonomic division. This study is continuing as we are developing new culturing techniques to cultivate maximal numbers of different prokaryotes from these soils. Physiological and biochemical characterization of these different isolates will then follow. Minimal soil disturbance has occurred; typically we use one gram of soil for each experiment.

Project title: **Microbiology of Hot Acid Springs**

Principal investigator: Dr. Gregory Olson
Phone number: 303-273-5697
Email: *lbl@rmi.net*
Address: 5902 McIntyre St.
Suite B
Golden, CO 80403

Objective: No work was done during 1999

Findings: No work was done during 1999

Project title: **Phylogenetic Analysis of High-Temperature Ecosystems**

Principal investigator: Dr. Norman Pace
Phone number: 303-735-1864
Email: *nrpace@colorado.edu*
Address: University of Colorado
Department of Molecular, Cellular and Developmental Biology
Campus Box 347
Boulder, CO 80309

Additional investigators: Carrine Blank, J. Kirk Harris, John R. Spear, Jeff Walker

Objective: Ongoing research within the park is founded on surveying microorganisms in various Yellowstone microbial ecosystems with varying solution chemistries. A molecular approach employing the analysis of the small sub-unit 16S rRNA ribosomal gene is used to determine what microbial members are present in these ecosystems. To accomplish this task, ongoing studies include analysis of both sub-aqueous and sub-aerial systems for bacterial, archaeal, and eukarial life.

Findings: Survey of microorganisms associated with geothermally produced siliceous sinters (e.g., Octopus Spring, Queens Laundry): Seven high-temperature (90-96° C) silica depositing springs throughout Yellowstone have been examined. In addition, the communities in different microenvironments (associated with spicular geyserite at the air-water interface and sub-aqueous strataform geyserite) have been compared in order to better understand how microorganisms may influence the deposition of silica. This work is in collaboration with Sherry Cady of Portland State University.

Survey of eukaryotes (eukaryotes are distinguished from bacteria and archaea by having a membrane bound nucleus) in anaerobic, low temperature environments: These anaerobic eukaryotes are displaying large diversity in the lower temperature environments, in the range of new genera to kingdoms. Such work with this domain of life that plants and animals are members of has never been done.

Novel Bacterial Lineages: Discovered the occurrence of novel bacterial phyogenetic divisions through molecular biological analysis from samples obtained within the park. A paper submitted to *Applied and Environmental Microbiology* describes two unique bacterial 16S rRNA gene sequences obtained from a dark green microbial mat on the northwest side of Black Hole in the White Creek Group. A third sequence obtained from the Obsidian Pool hot spring is also described. All three sequences have been deposited in GenBank. If accepted, the paper will be published in mid-2000. These sequences add to the greater body of knowledge about what types of bacteria occur in Yellowstone National Park.

High Temperature Limit of Life: Currently, the highest temperature limit for life is 113° C. A hot-water-filled, 250-foot deep well drilled in the 1960s in Biscuit Basin, Well Y-7, is currently being surveyed for the possible upper temperature limit of life. The well's water is 47° C at the surface. Glass slides and/or cotton fiber have been suspended at various depths down the length of the well on a stainless steel cable for varying amounts of time. We have found bacterial and archaeal cells up to 95° C. Ongoing work may push this temperature higher. The Park Service maintains the well under lock. The well is in a public location, requiring ranger assistance for both access and interpretation for this ongoing work.

Life in Cinder Pool, Norris Geyser Basin: Cinder Pool is a unique geothermal feature in the world within the Norris Geyser Basin. The pool has been geochemically well-described by others, but as yet, not biologically described. To do so, both cotton and fiberglass growth surfaces were hung in the 88° C Cinder Pool, at both the surface and two meters down, for approximately one month to allow for cellular adhesion. The fibers were removed and brought back to the lab for subsequent DNA extraction and molecular phylogenetic work-up. To date, with a limited number of molecular primers applied, a limited amount of diversity appears to be present. Such work will add to what is known about what kinds of microorganisms are capable of living in these extreme and unique environments.

Project title: **Exosporial Membrane Characteristics of Thermophilic *Clostridia***

Principal investigator: Dr. Barbara Panessa-Warren
Phone number: 631-444-3244
Email: *bwarren@epo.hsc.sunysb.edu*
Address: School of Health Technology and Management
State University of New York
Stony Brook, NY 11794-8200

Additional investigators: George T. Tortora, John B. Warren

Objective: To culture samples collected from Terrace Spring and Beryl Spring, isolate and subculture endospore-forming thermophilic bacteria. To identify the temperature at which growth is optimized. To characterize specific colonies by light microscopy, histochemistry, transmission and scanning electron microscopy.

Findings: We isolated seven different organisms based on colony type and temperature preference. All of the spores were found to have a ruthenium red-osmium positive outer covering (exosporium?), which may represent glycopeptide associated with attachment phenomena (glycocalyx). Extensive protein containing extracellular network attached some anaerobic species to one another and the substrate, effectively forming a tenacious matrix. This protein seems to be formed within the spore envelope and is liberated upon out-growth of the newly formed vegetative cell. Therefore, the newly released bacterial cells have a protective meshwork for attachment.

Project title: **Ecology of Microbial Phototrophs in Extreme Environments-Thermal and High Iron**

Principal investigator: Dr. Beverly Pierson
Phone number: 253-879-3353
Email: *bpierson@ups.edu*
Address: University of Puget Sound
Biology Department
1500 N. Warner
Tacoma, WA 98416

Additional investigators: Niki Parenteau, Victor Scopa, Kirstin Lightfoot

Objective: To determine the role of photosynthetic prokaryotes in thermal habitats and specifically in high iron thermal habitats. To determine the effects of iron on photosynthesis in cyanobacteria and anoxygenic phototrophs in hot spring mats.

Findings: Enrichment cultures have revealed evidence for the presence of purple photosynthetic bacteria that may be photoferrotrophs in the high iron sediments of Chocolate Pots Hot Springs. Very high iron stimulated photosynthetic rates in the *Synechococcus* and *Chlorofexus* mats suggest possible photoferrotrophy in the *Synechococcus* or *Chloroflexus*. Electron microscopy revealed that cyanobacteria are encrusted in iron in these springs. CLSM showed that filamentous prokaryotes bind and trap the iron sediments. A culture of *Chloroflexus* has been obtained from the highest temperature mats at Chocolate Pots. Preliminary experiments have shown no stimulation by iron in this culture. Work continues on the nature of iron stimulation of photosynthesis in the high temperature mats.

Project title: **Isolation and Characterization of Thermophilic Microorganisms**

Principal investigator: Dr. Robert Ramaley
Phone number: 402-559-6662
Email: *rramaley@unmc.edu*
Address: Department of Biochemistry
984525 University of Nebraska Medical Center
Omaha, NE 68198-4525

Objective: To determine the presence and ecological significance of the population of thermophilic microorganisms of the greater Yellowstone Ecosystem. To obtain additional thermophilic microorganisms for microbial and biochemical studies including the purificaion and characterization of thermostable enzymes.

Findings: A brief 1999 collection visit was made to Yellowstone (July 12-16) to obtain annual population samples from Obsidian Spring and nearby springs, and obtain several small "pink filament" samples from the runoff channel of Octopus Spring. The 1999 Obsidian samples again resulted in the isolation of a slowly growing, facultative anaerobic, spore forming bacterium which forms extremely swollen terminal spores (nicknamed doorknob). These isolates (*Moorella obsidium*) are similar (but not idential) to *Moorella glycerini* isolated from Calcite Springs and *Thermoaerobacter marianese* recently isolated from the world's deepest sea floor (Marian Trench). In additional to the usual aerobic microbial isolates from Obsidian, the 1999 samples also showed a modest level of isolates similar to *Thermomicrobium rosium*.

Project title: **Geochemical Constraints on the Ecology of the Deep Lineages within the Bacteria and Archaea**

Principal investigator: Dr. Anna-Louise Reysenbach
Phone number: 503-725-3864
Email: *reysenbacha@pdx.edu*
Address: Portland State University
Dept of Environmental Biology
1719 SW 10th Ave.
Science Building 2
Portland, OR 97207

Additional investigators: Everett Shock, Cristina Takacs

Objective: 1) Determine the microbial diversity and geochemistry associated with high temperature thermal springs in YNP. 2) Study the ecology of microbial communities inhabiting YNP thermal springs.

Findings: Our research in 1999 focused on Calcite Springs and Obsidian Pool. We collected extensive geochemical and molecular biological samples along chemical and physical gradients in the springs. Additionally, enrichment culture techniques were used to isolate novel thermophilic microorganisms. Initial results indicate that the geochemistry and community structure of the springs is dynamic on a spatial and temporal scale. Our research in 2000 will focus on linking geochemical and community differences and using our cultures to understand the physiological diversity of Calcite Springs and Obsidian Pool.

Project title:	**Analysis of Metal Resistance in Yellowstone Bacteria**
Principal investigator:	Dr. Frank Roberto
Phone number:	208-526-1096
Email:	*ffr@inel.gov*
Address:	Idaho National Engineering and Environmental Laboratory P.O. Box 1625 Idaho Falls, ID 83415-2203
Additional investigators:	Barrie Johnson, Simon Silver, Mark Delwiche, Heather Silverman

Objective: Identify and characterize heavy metal resistant bacteria from thermal features within YNP.

Findings: This year's sampling focused on the Norris area. Samples were obtained near Realgar Springs, north of the main Norris geyser basin, and south of the main Norris area near Tantalus Spring. Enrichments were performed to recover *Sulfolobus acidocaldarius* growing autotrophically. Only *Acidianus brierleyi* and *Metallosphaera sedula* were recovered under these conditions. It appears that recently published information indicating *S. acidocaldarius* is a heterotroph *only* may be correct. New techniques for cultivating *Sulfolobus* were obtained from Dr. Wolfram Zillig's laboratory in Germany, and are now being employed by our group and that of Dr. Mark Young at Montana State University. 16S rDNA analysis confirms physiological testing, but also indicates the possible presence of *Sulfolobus solfataricus* in the samples.

Project title: **Genetic Analysis of Brucella from Bison and the Generation of a PCR-Based Diagnostic System for Epidemiological and Ecological Studies**

Principal investigator: Dr. Rusty Rodriguez
Phone number: 206-526-6596
Email: *Rusty_Rodriguez@usgs.gov*
Address: USGS/BRD
6505 NE 65th
Seattle, WA 98115

Additional investigators: Regina Redman, Frank Roberto

Objective: The objectives of this work are to: 1) Determine the genetic complexity of *Brucella* isolates from a variety of animal hosts. 2) Develop a high sensitivity PCR-based diagnostic system to identify the presence of *Brucella* isolates. 3) Develop a PCR-based diagnostic system to track specific genotypes of the *Brucella* isolates. 4) Develop a PCR-based diagnostic system to discriminate live *Brucella* cells from dead cells. In addition to the objectives listed above, studies will be performed to convert the diagnostic systems to field adaptable systems capable of simple and rapid data generation.

Findings: We have completed the genetic analysis of *Brucella* isolates from several animal hosts, including bison, cattle, and elk. These data are currently being incorporated into a scientific manuscript. In addition, several PCR primer sets have been prepared that amplify products specifically from *Brucella abortus* isolates. Protocols have been developed for extracting *Brucella* cells from blood samples and detection using the PCR diagnostic system. This year, genotype-specific PCR primer sets will be generated for tracking isolates in the field and studies will begin to establish a diagnostic system to differentiate live and dead cells.

Project title: **Ecology, Physiology and Evolution of Microbes**

Principal investigator: Dr. Lynn Rothschild
Phone number: 650-604-6525
Email: *lrothschild@mail.arc.nasa.gov*
Address: Mail Stop 239-20
NASA Ames Research Center
Moffett Field, CA 94035-1000

Objective: The objective of this project is to study diurnal patterns of organismal physiology (e.g., photosynthesis, DNA synthesis) in order to better understand evolution on early Earth and the way organisms function in their environment today. Specifically, in 1999 the focus was on the effect of naturally occurring DNA damaging agents on DNA synthesis rates.

Findings: In 1999 research focused on the effects of UV radiation and hydrogen peroxide on microbial mat communities in the Norris Geyser Basin. We found that UV radiation enhances DNA synthesis rates during the day, which we interpret as being indicative of excision repair. However, previous work suggests that the damage may be due to UVA effects mediated through oxidative damage rather than the direct effect of UVB. Experiments adding hydrogen peroxide to samples, done in collaboration with Cindy Wilson, showed an increase in DNA synthesis in response to small amounts of additional hydrogen peroxide, and a decrease in response to high levels. *Zygogonium* mats that were placed under UV opaque screens from July to September showed a down regulation in DNA synthesis when finally exposed to solar radiation in contrast to an adjacent mat that had been left exposed to full solar radiation. These studies will be repeated and extended in 2000.

Project title:	**Isolation, Identification, and Characterization of Microorganisms Living in Extreme Environments**
Principal investigator:	Dr. Perry Russell
Phone number:	606-539-4388
Email:	*prussell@cc.cumber.edu*
Address:	Cumberland College Department of Biology 7196 College Station Drive Williamsburg, KY 40769

Objective: There are two main project objectives. One is the training and inspiring of undergraduate students in the field of microbiology. Secondly, identification and characterization of the many unidentified and novel microorganisms associated with the thermal features in Yellowstone National Park.

Findings: There have been no further results from this study for the year 1999. Two morphologically different bacterial colonies initially grew on a very minimal agar media at higher temperatures. However, by the time these samples were returned to the laboratory in Kentucky for further characterization, the organisms were no longer growing and there were no survivors. However, I was able to obtain one of my objectives by having a student with me who was able to observe and learn something about sampling techniques and laboratory techniques in molecular biology. All of the samples I obtained from the park were consumed and I will return again in the summer of 2000 to obtain more samples.

Project title: **Subsurface Transport Mechanisms for High-Temperature Microbial Life and the Nature of the Subsurface Biosphere**

Principal investigator: Dr. Micheal P. Ryan
Phone number: 703-648-6770
Email: *mryan@usgs.gov*
Address: 926 National Center
U.S. Geological Survey
12201 Sunrise Valley Drive
Reston, VA 20192-0001

Objective: Determination of the transport mechanisms and the three-dimensional structure of thermophilic and hyperthermophilic microbial communities. The work is done in relationship to the structure(s) of the thermal intervals and isotherms of their optimal temperature range and limiting temperatures.

Findings: Finite-element numerical simulations have been completed for the determination of the flow velocity vectors and the thermal structure of theoretical end-member microbial transport scenarios. These include: 1) continuous convective transport of microbes in fracture and microporous volcanic rock; 2) continuous convective transport in multiply-fractured volcanic rock.

Project title: ***Pantoea Agglomerans* and Other Epiphytic Bacteria of Grasses in Yellowstone National Park**

Principal investigator: Dr. David Sands
Phone number: 406-994-5151
Email: *uplds@montana.edu*
Address: Department of Plant Sciences
Montana State University
Bozeman, MT 59717

Additional investigators: David Pascual, Alice Pilgeram, Tim Anderson, Laura Carsten

Objective: Vaccinating mammals to induce immunization has proven to be effective in the past when using common syringe and needle methods. However, with the increasing threat of feral animals transmitting disease to domestic livestock, novel vaccine delivery systems may be more effective for inoculation of wild species in their natural environment. Brucellosis is a disease that causes fetal abortions and, according to serological testing, infects 48% of bison and 1% of elk in Yellowstone National Park and 76% of bison and 50% of elk in neighboring Grand Teton National Park. *Brucella abortus* poses an economic threat to the U.S. livestock industry. Although interspecies transmission of Brucellosis in nature has yet to be proven, the phenomenon has been demonstrated in laboratory settings. The implications are significant because, if even a single cow is infected, slaughter of all *B. abortus* reactive

animals in the herd is mandated - regardless of whether they actually have the disease. This potential loss could significantly impact Montana's economy. This research project is focused on adapting common plant epiphytic bacteria to carry antigens that will confer immunity to brucellosis via the mucosal route.

Findings: Grass samples from several areas in northern Yellowstone park were screened for commonly occurring epiphytes throughout the summer. Several common species of bacteria were identified using this method. Of these, five were chosen as test species (*Pantoea agglomerans*, *Pseudomonas putida*, *Rahnella aquatilis*, *Hafnia alvei*, and *Pseudomonas syringae* pv. syringae). Currently, little is known about the specific immune responses elicited by bison. However, the protein K99 is known to stimulate mucosal immunity in these animals. The five species of bacteria listed above were genetically modified to express the K99 protein. Currently, tests are underway to confirm that these modified bacteria can still compete with native bacteria found on leaf surfaces. In addition, mice have been dosed with the modified bacteria to determine the efficacy of the strategy.

Development of this novel delivery system has promising implications beyond bison inoculation. The system could provide an inexpensive way to inoculate domestic cattle against a wide range of diseases by spraying the product on hay or pasture grasses. This project is one of the first attempts to utilize microorganisms to immunize animals in their natural environment.

Project title:	**Diversity and Habitat Range of Sulfate-Reducing Microorganisms**
Principal investigator:	Dr. David Stahl
Phone number:	847-491-4997
Email:	*d-stahl@nwu.edu*
Address:	Northwestern University Department of Civil Engineering 2145 Sheridan Road Evanston, IL 60208-3109
Additional investigators:	David Ward, Nancy Hinman, Susan Fishbain, Alakendra Roychoudhury, Brad Jackson

Objective: We have analyzed samples from a number of different regions throughout the park. Sites include Bath Lake Vista, New Pit Spring, and Roland's Well Spring within the Mammoth Hot Springs region; Octopus and Mushroom Springs; two sites at the Nymph Creek area denoted as Nymph Creek and Black Sediment Pool; four sites in the Washburn region denoted as Site A, B, Acid Inkpot and Inkpot; five sites at Norris 100 Springs Plain denoted as Sites C, D, E, Cinder and Black Spring; and Obsidian Pool and Moose Pool in the Mud Volcano area. These sites provide a wide range of temperature and pH gradients (38°-90° C; pH 2-8). Significant rates of reduction were observed at Site C, Obsidian Pool, Nymph Creek, and Black Sediment Pool. We have established sulfate-reducing enrich-

ment cultures from the microbial mats of the Mammoth Hot Springs region. Stable enrichments were developed from New Pit Spring (H_2/acetate as substrates) and from Bath Lake Vista (acetate) and Roland's Well Springs (acetate). The DSR genes were amplified from each of the enrichment cultures using the DSR1F-DSR4R primer pair and cloned (pCRII vector) for sequence determination. We have also initiated enrichments from Obsidian and Black Sediment pools that demonstrate sulfate-reducing activity. We are currently analyzing these enrichments to determine if the organisms active in laboratory cultures are comparable to those identified by direct sequencing.

Findings: Our research at Yellowstone National Park has focused on better defining the diversity of sulfate-reducing bacteria along environmental gradients of pH and temperature. Organisms having the capacity to respire sulfate drive a key step in the global cycling of sulfur and are likely an important biological presence in many of the sulfur-rich geothermal areas within Yellowstone National Park. A long-term objective is to better define the environmental limits of dissimilatory sulfate reduction. Our primary method of assessing population diversity has been comparative sequence analysis of the highly conserved dissimilatory sulfite reductase (DSR) gene. This gene can be selectively amplified from DNA recovered from site material using PCR, as reported by our research group. Comparative sequencing of cloned DSR genes avoids the usual biases associated with culture-based methods of characterization. We have complemented this molecular characterization with on-site measurements of sulfate respiration in order to relate gene presence to corresponding environmental activity. We are also using more traditional culture-based methods to analyze cultivable sulfate-reducing bacteria. The recovery of deeply-diverging phylogenetic lineages, as defined by DSR gene sequence divergence, suggests that our current understanding of this important functional group of microorganisms is incomplete. Our combined analyses suggest that sulfate respiration is an important biogeochemical process in many of Yellowstone's geothermal features.

Project title:	**Isolation of New Hyperthermophiles and Investigations of Hyperthermophilic Bioptopes**
Principal investigator:	Dr. Karl Stetter
Phone number:	941-943-3160
Email:	*Karl.Stetter@biologie.uni-rege*
Address:	Universitaet Regensburg Universitaetsstrasse 31 93053 Regensburg Regensburg, Germany 93053
Additional investigators:	Robert Huber, Wolfgang Eder, Gudrun Amann, Manuela Baumgartner

Objective: Isolation of new hyperthermophiles and investigations of hyperthermophilic biotopes.

Findings: A member of the novel kingdom Korarcheaota, identified by 16S rRNA gene sequence

analysis, collected from Obsidian Pool, has been grown in a continuous lab culture at 85° C in our institute. Comparative PCR amplifications of SSU rRNA gene sequences from this culture indicated substantial preferential PCR amplification of pJP27 sequences with korarchaeote-specific PCR primers.

Project title:	**Integrated Biogeochemical Database**
Principal investigator:	Dr. Daphne Stoner
Phone number:	208-526-8786
Email:	*dstoner@inel.gov*
Address:	Idaho National Engineering and Environmental Laboratory P.O. Box 1625 Idaho Falls, ID 83415
Additional investigators:	Ronald Rope

Objective: The objective of this multiple year project is to develop an integrated relational database and Geographic Information System (GIS) for mapping biodiversity data and associated geochemical and hydrological attributes in extreme environments.

Findings: The prototype data management system was completed and launched on the Internet. The database for housing and managing field and reported (citations) data for thermophilic microorganisms and geyser/hot springs characteristics was revised. Fields for temporal and spatial (mapping) data were developed as well as links to photographs and output field forms for field data and query reports. Draft standard field protocols and safety analysis for characterizing thermal features were developed.

Project title:	**Development of Harsh Environment Biosensors**
Principal investigator:	Vicki Thompson
Phone number:	208-526-8833
Email:	*thomvs@inel.gov*
Address:	Idaho National Engineering and Environmental Laboratory P.O. Box 1625 Idaho Falls, ID 83415-2203
Additional investigators:	Diane Key, William Apel, William Keener, Frank Roberto

Objective: The objective of this project is to culture thermophilic microorganisms from Yellowstone hot springs. These organisms will then be tested for the presence of various enzymatic activities and the enzymes will be isolated and purified from organisms that show large amounts of activity. The enzymes will be studied to determine how high temperatures affect their characteristics and compared to low temperature versions of the enzymes.

Findings: Two sampling trips were taken to YNP this summer on June 9 and July 21. Water and microbial mats were collected from Firehole Pool, Twin Vista Butte Springs, Octopus Springs, Spring LNN2, and from the Hot Lake area. The samples were inoculated into liquid and solid minimal salts medium containing lactate as the carbon source and incubated at 70° C for up to four weeks. Nineteen organisms were isolated from the samples and characterized as Gram + and - rods. Additional samples were incubated under denitrifying conditions with collaborators at Washington State University. No organisms were isolated under those conditions. Samples were inoculated into minimal media containing amides and ammonium nitrate. Three uncharacterized strains have been isolated under these conditions.

Project title: **Search for the Upper Temperature Limit of Eukarotic Life**

Principal investigator: Dr. Jonathan Trent
Phone number: 650-604-3686
Email: *jtrent@mail.arc.nasa.gov*
Address: NASA Ames Research Center
Mail Stop 239-4
Moffett Field, CA 94035

Additional investigators: Susanne J. Trent, Fred Martwick, John Hines

Objective: Search for multi-cellular organisms living at high temperatures using video cameras equipped with macro-lenses and configured to hold "bait" in view with the objective of attracting these organisms into the field of view. This search requires meeting the following objectives: 1) Construct a compact video camera system that can cope with high temperatures (up to 90° C) and pH down to 1.0. 2) Assemble cameras into an array that will allow the temperature, pH, and depth to be monitored in the vicinity of the camera. 3) Field test the system for deployment in remote sampling areas (transported in backpacks).

Findings: Our first year saw the successful construction of a video camera system that was field tested at a variety of sites in Yellowstone National Park. Two small video cameras built by DeepSea Power and Light (normally used for investigating plumbing) were capable of withstanding temperatures of up to 120° C for one hour with noticeable, but acceptable degradation of the image. A probe, consisting of two cameras (one macro and one wide angle), lights on each camera, a versatile bait holder, and temperature, pH, and depth sensors, was fabricated out of Delran. The probe has 30 meters of cable and was supported by a data acquisition system attached to a backpack frame. The complete system weighed about 150 lbs. and could be carried by three hikers with large backpacks. It was deployed at a variety of sites in Yellowstone, ranging from Rabbit Creek (32° C) to Cinder Pool (120° C). Cameras and depth sensor worked well but the temperature and pH sensors failed. In Rabbit Creek, we observed Caddisfly larvae (*Heliopsyche borealis*), fly larvae (blood worms), and ostracods. Underwater video of hot springs was made available and televised on national television.

Project title: **Ecology of Hot Spring Microbial Communities**

Principal investigator: Dr. David Ward
Phone number: 406-994-3401
Email: *umbdw@montana.edu*
Address: Department of Land Resources and Environmental Sciences
P.O. Box 173120
Montana State University
Bozeman, MT 59717

Additional investigators: Mary Bateson, Thane Papke, Mike Ferris, Uli Nuebel

Objective: The general objective of our research is to understand the distribution and activity of microorganisms inhabiting microbial mat communities in geothermal effluents. At the moment, we are particularly interested in understanding the composition, structure, and physiology of these mat communities, as models of microbial communities in general. We are using ribosomal RNA (rRNA), intervening transcribed spacer (ITS), and lipid biochemical cell components to identify community members. Our work relates to evolutionary microbiology in the sense that these gene sequences give phylogenetic information, and the association of lipids with their microbial sources helps us interpret the chemical fossil record produced by organic geochemists. In addition, we are attempting to evaluate whether the stable carbon isotope ratios of specific lipid biomarkers might help distinguish modern mat communities constructed by either cyanobacteria or green nonsulfur bacteria and hence their stromatolite counterparts in the fossil record.

Findings: During 1999, we made the following major observations: Cyanobacteria: *Synechococcus* populations in Mushroom Springs show a similar temperature distribution to that in Octopus Springs. We are currently examining their vertical distribution at four temperature-defined sites where we characterized light and chemical parameters using microsensors (Kühl). We succeeded in cultivating 5 to 10% of the *Synechococcus* cells that are apparently genetically dominant populations and are pursuing the rest as well as studying their adaptations to light and temperature. We have demonstrated that genetically unique *Synechococcus* occur in hot springs around the world and even within Yellowstone.

Green nonsulfur bacteria: We used rRNA probes to demonstrate that the organisms contributing type-C 16S rRNA sequences in mat communities are filamentous and we are proceeding to evaluate their metabolisms through combined probing and microautoradiography and/or microscope spectrophotometry. We have continued experiments to evaluate the autotrophic metabolism of green nonsulfur bacteria by 1) using microsensors (Kühl) to demonstrate that the potential electron donors hydrogen and sulfide occur in the photic zone of mats containing cyanobacteria and green nonsulfur bacteria (i.e., Mushroom Springs and Tangerine Springs) in the morning and evening; and 2) conducting further 13C labeling studies at such times of day.